Structural determination of archaeal UDP-*N*-acetylglucosamine 4-epimerase from *Methanobrevibacter ruminantium* M1 in complex with the bacterial cell wall intermediate UDP-*N*-acetylmuramic acid

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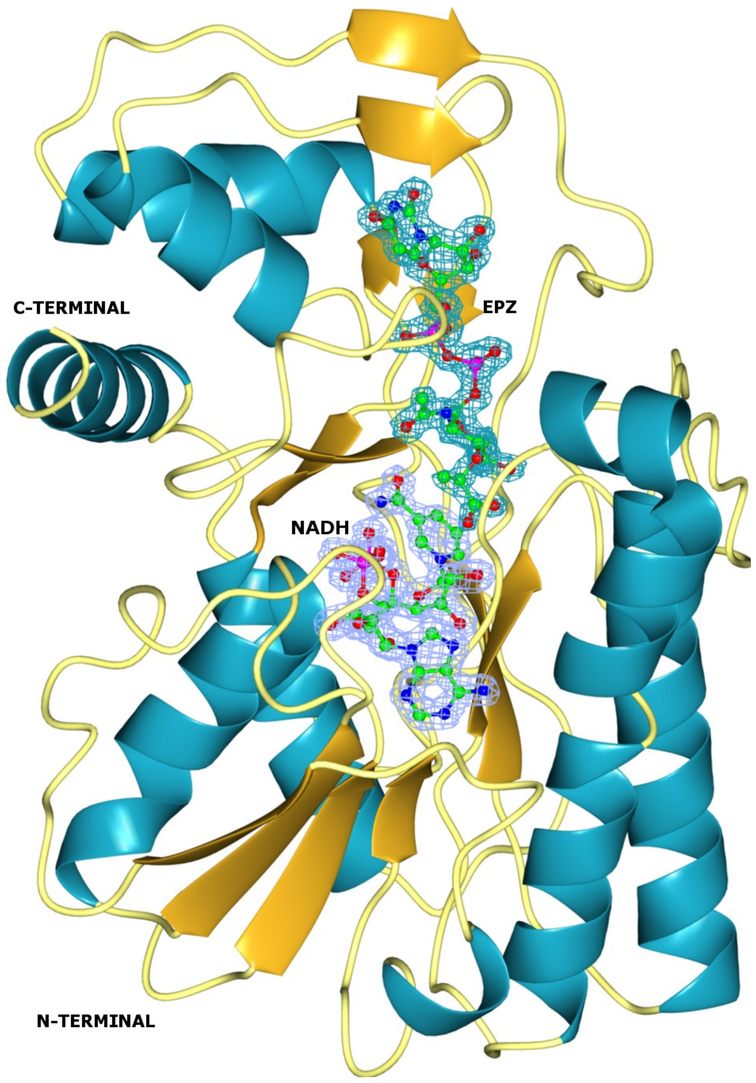
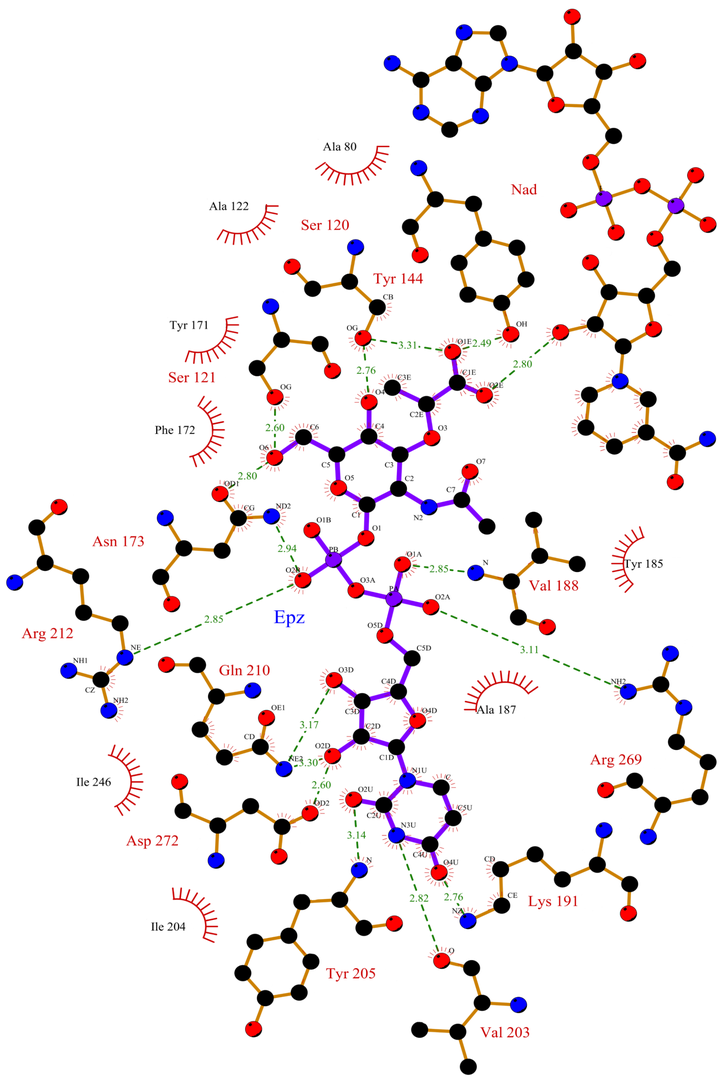
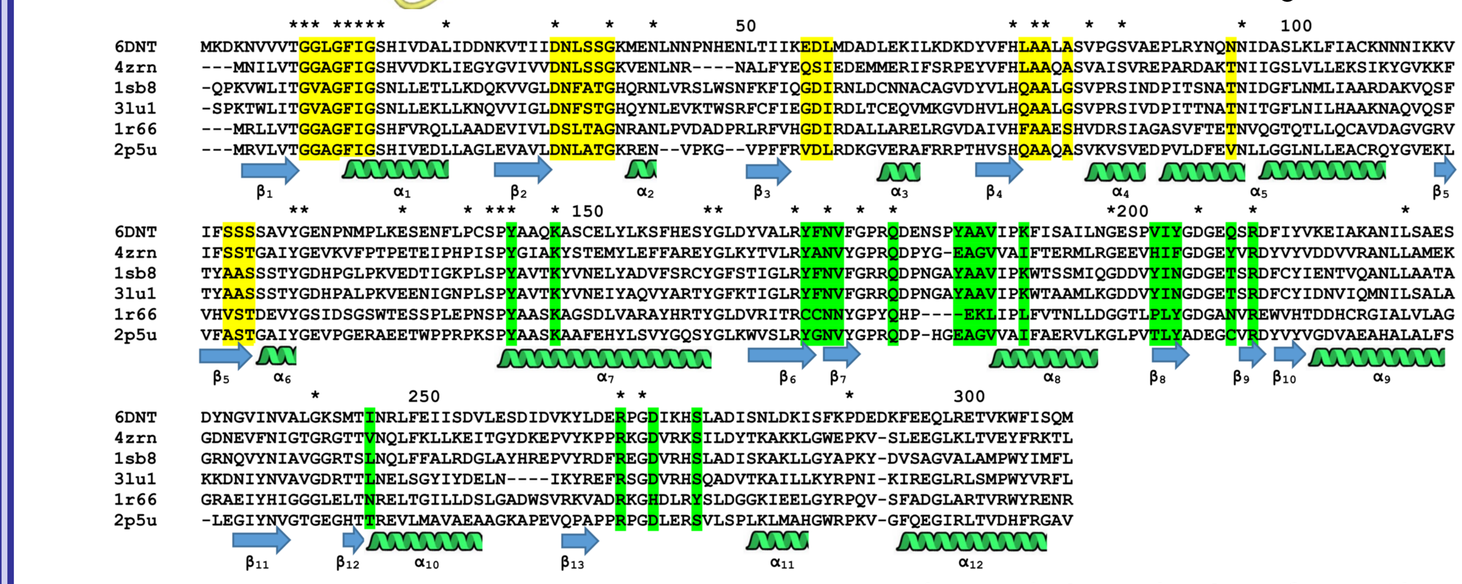
Abstract: The crystal structure of UDP-*N*-acetylglucosamine 4-epimerase (UDP-GlcNAc 4-epimerase; WbpP; EC 5.1.3.7), from the archaeal methanogen *Methanobrevibacter ruminantium* strain M1, was determined to a resolution of 1.65 Å. The structure, with a single monomer in the crystallographic asymmetric unit, contained a conserved N-terminal Rossmann fold for nucleotide binding and an active site positioned in the C-terminus. UDP-GlcNAc 4-epimerase is a member of the short-chain dehydrogenase/reductase superfamily, sharing sequence motifs and structural elements characteristic of this family of oxidoreductases and bacterial 4-epimerases. The protein was co-crystallized with coenzyme NADH and UDP-N-acetylmuramic acid, the latter an unintended inclusion and well known product of the bacterial enzyme MurB and a critical intermediate for bacterial cell wall synthesis. This is a non-native UDP sugar amongst archaea and was most likely incorporated from the *Eschericha coli* expression host during purification of the recombinant enzyme.

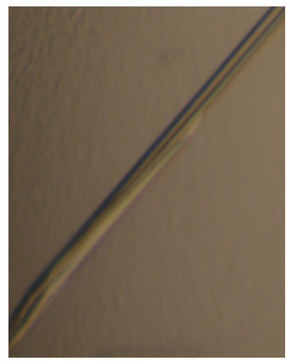
Crystallisation and x-ray diffraction

M1 WbpP was expressed in *E. coli* Rosetta 2 (DE3) cells and purified from cell extracts using nickel-affinity chromatography. The enzyme (11.3 mg/ml) was crystallised using the JCSG-plus screen in buffer containing 0.2 M NaCl, 0.1 M Bis-Tris pH 5.9 and 25% (w/v) PEG 3350. Diffraction data was collected at the Australian Synchrotron MX2 beamline.









Space group	$P\,3_1\,2\,1$
Unit Cell a, b, c (Å)	89.50, 89.50, 82.72
α, β, γ (°)	90.0, 90.0, 120.0
Wavelength (Å)	0.95370
Resolution Range (Å)	41.36-1.65 (1.68-1.65)
R_{pim}*	0.026 (0.248)
Completeness (%)*	97.8 (56.9)
Multiplicity*	12.9 (10.4)
I/σ(I)*	22.6 (3.4)
R_{cryst} (%)/R_{free} (%)	15.20/17.20
Matthews coeff. (Å^3 Da^{-1})	2.48

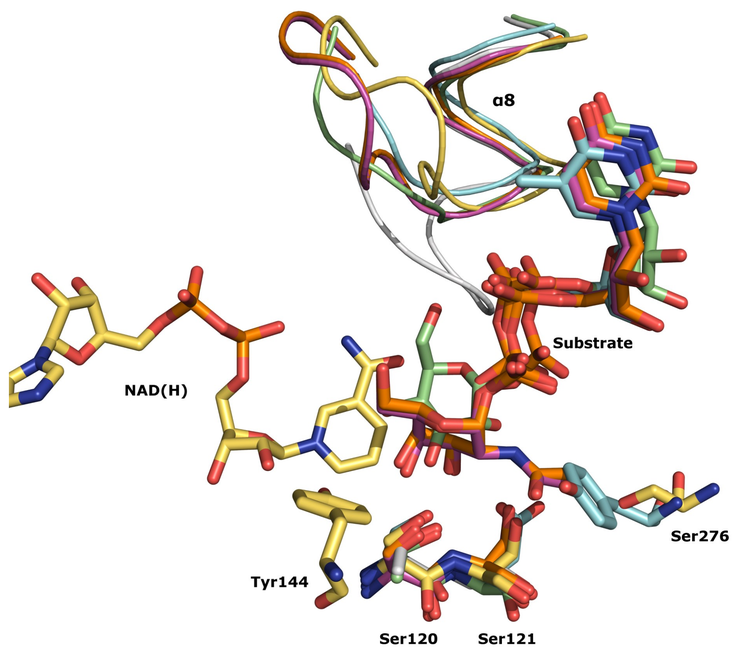
Structural architecture of the UDP-GlcNAc 4-epimerase monomer

The ternary complex was achieved serendipitously as no coenzyme or substrate was intentionally added.

Pictured far left is a ribbon representation of *Methanobrevibacter ruminantium* UDP-GlcNAc 4-epimerase with bound coenzyme (**NAD** in **blue**) and tightly bound bacterial sugar (**EPZ** in **cyan**). The observed electron density map (**2Fo-Fc**) for the bound molecules is contoured at **1.0-σ**.

The monomer presents two distinct folds/domains common amongst archaeal, bacterial and eukaryotic 4-epimerases, and sequence and structural characteristics that belong to enzymes of the larger super family of short-chain dehydrogenase/reductases incorporating a series of seven parallel β-strands (**β1** – **β6** and **β11**) and eight alpha helices (**α1-α7** and **α9**).

A modified PDBsum depiction of the binding site residues within 4 Å of EPZ are shown. Hydrogen bond interactions are depicted as dotted lines.



The catalytic mechanism of UDP-GlcNAc 4-epimerase

A Dali-lite pair-wise structural alignment (left) and structural superimposition (right) of *M. ruminantium* UDP-GlcNAc 4-epimerase (6DNT; yellow) reveals a distinct homology with bacterial UDP-glucose and UDP-GlcNAc 4-epimerases such as 4ZRN (*Thermotoga maritima*; green), 1SB8 (*Pseudomonas aeruginosa*; purple), 3LU1 (*Plesiomonas shigelloides*; orange), 1R66 (*Streptomyces venezuelae*; blue) and 2P5U (*Thermus thermophilus*; white). On the left cofactor and substrate binding residues of UDP-GlcNAc 4-epimerase are highlighted in yellow and green, respectively. Identical residues are indicated with a *, β-strands with blue arrows and α-helices with green helices. On the right α8 is shown in ribbon form and we identify the position of the catalytic tyrosine (at Tyr144), sugar binding residues (located at the Ser120 and Ser121 positions) and the "gatekeeper" residue (the Ser276 position). Each enzyme possesses an identical orientation for the coenzyme, the catalytic tyrosine and gatekeeper serine of the active site. Variability in orientation of α8 and the substrate binding loop across the enzymes is highlighted, as are the differences of sugar binding residues and the presence of a tyrosine at the "gatekeeper" position in 1R66.

References – This work was published in Carbone, V., Schofield, L., Sang, C., Sutherland-Smith, A and Ronimus, RS (**2018**). Structural determination of archaeal UDP-*N*-acetylglucosamine 4-epimerase from *Methanobrevibacter ruminantium* M1 in complex with the bacterial cell wall intermediate UDP- *N* -acetylmuramic acid. Proteins: Structure, Function, and Bioinformatics. 10.1002/prot.25606.

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